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TITLE: On the Pathophysiology of Cutaneous Café-au-lait Lesions in Neurofibromatosis and the Role of Keratinocyte and/or Fibroblast-Synthesized Cytokines

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14. ABSTRACT:

Fibroblasts derived from the CALM lesion of NF1 are capable of inducing melanin synthesis by normal human melanocytes as demonstrated in both an in vitro and in vivo model system suggesting that undefined cytokines are synthesized by NF1 fibroblasts putatively resulting in CALM.

15. SUBJECT TERMS
Skin, café-au-lait, pigmentation, melanocytes

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INTRODUCTION

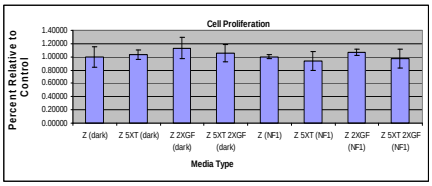
Mutations at the *NF1* locus predisposes neurofibromatosis type 1 INF1) patients to skin pigmentary abnormalities: hyperpigmented café-au-lait macules (CALM) and axillary & inguinal freckling. The pathophysiology of these *NF1*-related abnormalities has yet to be elucidated. Much controversial data exists pertaining to the etiology of these pigmentary lesions, however the consensus suggests that the melanocyte population in CALM is hyperactive resulting in increased pigment synthesis. However, melanocytes cutured in isolation from skin biopsies of NF1 patients demonstrated no, or at most minimal, increase in melanization. This suggests that extracellular factors (i.e., cytokines) in the NF1 skin may be the important elements inducing hyperactivity of the melanocytes resulting in CALM. We proposed that an NF1 mutation, and/or loss of heterozygosity, causes aberrant production of secretory cytokines by the NF1 fibroblast of the skin that ultimately influences the melanocytes resulting in CALM. This study was designed to understand the etiology of CALM in NF1 by assessing interactions between melanocytes (M) and fibroblasts (Fb) in cultured derived from NF1 patients.

BODY

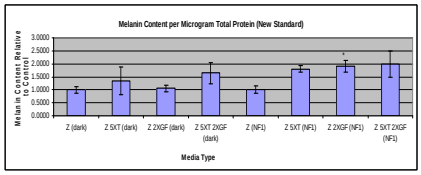
To test the hypothesis proposed above, we initially assessed the proliferation and melanization of cultured M derived from CALM-lesional sites and normal skin under normal and promotional (i.e., increased growth factors) conditions. Subsequently we assessed serum-free, conditioned media from cultured Fb derived from CALM-lesional sites in comparison to Fb derived from normal individuals, for the ability to stimulate proliferation and melanogenesis on cutlured normal M as well as melanocytes derived from CALM-lesional sites in several patients with NF1.

KEY RESEARCH ACCOMPLISHMENTS

Proliferation and response to growth factors were similar between NHM and NF1 melanocytes. – Melanocyte cultures were developed from 5 patients with NF1 and 5 unaffected individuals. Proliferation of cultures were assess in media with 1, 2 or 5 X growth factors (GF) added. No statistical differences were observed.

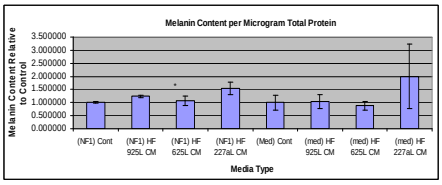


Induction of melanization by growth factors was enhanced in NF1 melanocytes compared to NHM. – Cultures described immediately above were assessed for melanin content. NF1 melanocytes exhibited a statistically significant increase in melanin by GF.



Proliferation in response to conditioned media from NF1-fibroblasts was similar between NHM and NF1 melanocytes. – Fibroblasts cultures were developed from 5 patients with NF1 and 5 unaffected individuals. Conditioned media from these cultures was then combined with normal melanocyte growth media at various ratios for 10 days and proliferation subsequently assessed. No significant differences were observed among the various groups (data not shown).

Induction of melanization by conditioned media from NF1-fibroblasts was enhanced in NF1 melanocytes compared to NHM. Cultures described immediately above were assessed for melanin content. NHM exhibited a statistically significant increase in melanin induced by NF1 derived Fb as oppose to normal Fb.



CONCLUSION

Fibroblasts derived from the CALM lesion of NF1 are capable of inducing melanin synthesis by normal human melanocytes as demonstrated in an *in vitro* model system suggesting that undefined cytokines are synthesized by NF1 fibroblasts putatively resulting in CALM.